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Signal Amplification: Let's Turn Down The Lights

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G protein activation by membrane-bound receptors initiates a chain reaction that amplifies cellular responses to external signals. In retinal photoreceptors, amplification by the phototransduction cascade is regulated by quickly switching off the visual pigment that acts as the receptor for light.

Cells respond to an enormous variety of external signals, such as hormones, neurotransmitters, odours and light. The reliable sensing of weak signals requires that they be amplified. A very common strategy involves a 'chain reaction' triggered by a receptor that spans the membrane seven times and interacts with a G protein [1,2]. There are hundreds of seven-helix receptors, each specialized for a particular task, such as the β-adrenergic receptor which becomes active on binding epinephrine, or the visual pigment rhodopsin which absorbs light in rod photoreceptors. Recent evidence indicates that the active lifetime of a seven-helix receptor is one of the key factors setting the amplification of these signalling cascades [3].

Amplification in a G protein Cascade

The basic strategy for signal amplification in the phototransduction cascade is shown in Figure 1 [4]. The receptor, rhodopsin, becomes active on absorbing a photon of light and in this active state switches on the G protein, transducin, which in turn activates the effector enzyme, cGMP phosphodiesterase (PDE). In rod photoreceptors, these reactions occur on internal membranes. G proteins are in the OFF state when the α subunit is occupied by GDP and ON when it is bound to GTP. The active receptor flicks ON the G protein switch by catalyzing the replacement of GDP by GTP. The α subunit of transducin (T_α) can then dissociate from the βγ dimer and activate the PDE. The first step in the cascade provides amplification because a single rhodopsin molecule can diffuse in the membrane and activate hundreds of transducin molecules [5].

The second step does not provide amplification, because a single T_α activates a single PDE molecule. But the PDE has a very high activity and rapidly hydrolyzes cGMP, the internal messenger molecule. The phototransduction cascade in rods can detect the weakest possible signal — a single photon — because the fall in cGMP concentration triggered by photoisomerization of a single rhodopsin leads to the closure of hundreds of cation channels in the surface membrane [4]. The electrical signal generated by a single photon has the form shown in Figure 2. A second example of an effector enzyme is adenylyl cyclase, which produces cAMP when the β-adrenergic receptor activates the stimulatory G protein G_s [1,6].

A potential problem with a cascade that has high amplification is that the response can saturate if the signal is strong. The way round this problem is to reduce the amplification according to the size of the response that has already been generated. In photoreceptors, this feedback is provided by the free calcium concentration in the outer segment. When cGMP-gated channels are closed by light, the calcium concentration falls, causing calcium-sensitive proteins to reduce the amplification of the cascade [7–9]. Our understanding of the molecular basis of phototransduction in rods has helped us understand the basic design features of G protein cascades [1,2]. We now have a detailed understanding of the reactions governing the activation of the response and recent attention has focused on how G protein cascades are switched off again [4].

Switching OFF The Cascade

The G protein switch is turned OFF when the intrinsic GTPase activity of the α subunit hydrolyzes GTP to GDP (Figure 1). As a result, the α subunit falls off the effector enzyme, which returns to its inactive state. But switching off the G protein is not enough — the active receptor must also be turned off to prevent it from activating other G protein molecules on the membrane. In rods, rhodopsin deactivation is a two-step process. First, rhodopsin is phosphorylated by rhodopsin kinase. Then arrestin binds to the phosphorylated receptor, quenching its activity. The β-adrenergic receptor is also turned off by a specific kinase and quenching protein.

An outstanding feature of the deactivation process in rods is its reproducibilty. Usually, the active lifetime of a single molecule varies widely from trial to trial, but the duration of rhodopsin's activity is suprisingly constant, leading to an electrical response that varies little in amplitude or duration [10]. Mel Simons, Denis Baylor and colleagues [11,12] have investigated how G protein cascades are controlled using a combination of transgenic technology with electrophysiology in mouse photoreceptors. Removing all six phosphorylation sites in mouse rhodopsin blocks the switching off of the response to light [11]. Removing just one or two phosphorylation sites makes the responses to a single photon less reproducible [12].

After a flash, PDE activity falls with a time-constant of about 200 milliseconds in mouse rods [13] and about 2 seconds in amphibian rods. Which is the slow reaction that limits the rate of recovery: deactivation of rhodopsin or GTP hydrolysis by transducin? One approach to this question has been to record the light response in a single amphibian rod after opening up one end of the outer segment to allow manipulation of the phototransduction cacscade. Rieke and Baylor

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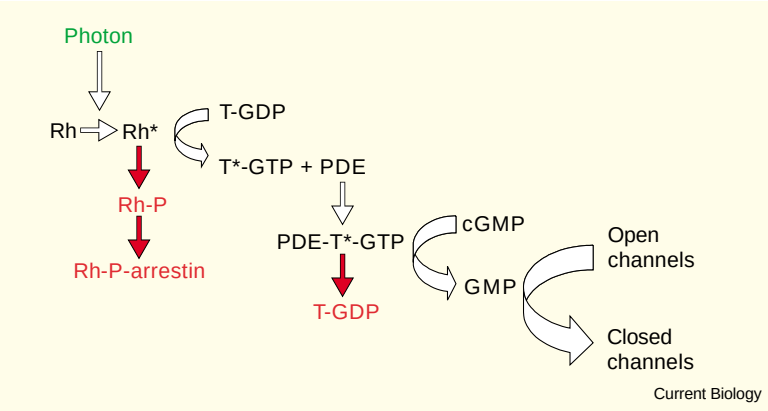

Figure 1. Phototransduction cascade in rods and cones: an archetypical G-protein signalling cascade.

Reactions involved in activation of the response are shown in black and deactivation reactions in red. Reactions providing amplification are depicted by bold arrows. Rh*; active rhodopsin. T*; transducin in the active state. PDE; cGMP phosphodiesterase.

[10] found that applying GTP during recovery of the response to a flash increased the response, suggesting that active rhodopsin molecules were still present at these long delays, and that rhodopsin deactivation governed the rate of recovery.

The opposite conclusion was drawn from experiments in which transducin deactivation was blocked by replacing GTP with GTP-γ-S (which cannot be hydrolyzed). If GTP hydrolysis by transducin is indeed fast compared to rhodopsin deactivation, many more transducin molecules would be expected to stay active after binding GTP-γ-S and so the response generated by a flash should become larger. In fact, GTP-γ-S did not alter the size of the response to a dim flash, indicating that very little GTP hydrolysis occurred during the time that rhodopsin molecules were active [14]. This suggests that rhodopsin deactivation is in fact much faster than GTP hydrolysis by transducin. Furthermore, reducing the calcium concentration at the time of the flash accelerated the deactivation of rhodopsin and so reduced the amount of PDE activated [14]. Calcium regulates the active lifetime of rhodopsin through recoverin, a calcium-binding protein that inhibits rhodopsin kinase in the calcium-bound state [15,16].

Elegant support for the idea that rhodopsin deactivation is relatively fast was provided by experiments in mutant mice lacking RGS9-1, a protein that accelerates GTP hydrolysis by transducin [13]. Responses to single photons recovered 40 times more slowly, yet the amplitudes of the responses were barely affected (Figure 2). Further, these responses could be modeled assuming that rhodopsin deactivation is fast and that GTP hydrolysis by transducin limits recovery.

Nature's Experiment: Rods Versus Cones

The effects of inhibiting deactivation of transducin suggest a simple rule for regulation of responses in rods: the active lifetime of the receptor sets the overall amplification of the cascade, while deactivation of the G protein sets recovery time [14]. Is this a general rule for G protein cascades? Nature has provided us with an interesting way of approaching this question. The second class of photoreceptor in the verteberate retina, the cones, are at least 30-fold less sensitive than rods and respond to a flash of light much more briefly. Although each element of the phototransduction cascade comes in a version specific to a rod or cone, the basic scheme is the same. The design principles of the cascade in rods predicts that response recovery is faster in cones because cone transducin hydrolyzes GTP faster. An indication that this might be the case is provided by the observation that the GTPase-activating protein RGS9-1 is expressed at higher levels in cones than in rods [17].

A second prediction is that the lower amplification in cones is due to faster deactivation of the visual pigment. Satoru Kawamura's group [3] has now directly compared the activation of PDE in purified rod and cone membranes. They found that the number of transducin molecules activated per absorbed photon was about 30-fold lower in cone membranes. This reduced amplification was paralled by a striking difference in the phosphorylation of receptors after photon absorbtion: the visual pigment in cones was phosphorylated at least 20-fold faster than rhodopsin in rods. It therefore seems that the lower amplification in cones is largely because the pigment is deactivated more rapidly, so that fewer G proteins are switched ON. This result therefore provides further support for the idea that the amplification of the phototransduction cascade is set by the active lifetime of the receptor, rather than the rate of GTP hydrolysis by the G protein.

Why have fast rhodopsin deactivation control the amplitude of the light response and slow transducin deactivation control recovery? Having the receptor and G-protein deactivated sequentially rather than simultaneously provides an important advantage — energetic efficiency. In this scheme there is a minimum of GTP hydrolysis because a fixed number of transducin molecules become activated to achieve a given level of PDE activity and these deactivate after the response has reached its peak [14]. Imagine if transducin deactivation were the faster reaction: at the peak of the response transducin molecules would be continuously switching on and off as they were activated by rhodopsin and then deactivated by GTP hydrolysis i.e transducin molecules would be turning over to achieve a given level of PDE activity. It will be interesting to see whether gain control at the receptor and time-scale control at the G-protein are general features of G-protein mediated responses.

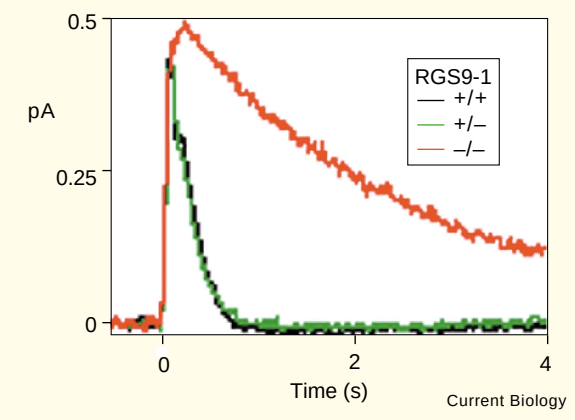

Figure 2. Single photon responses of individual mouse rods.

Averaged single photon responses were recorded from individual mouse rods using a suction electrode to collect the current flowing through cGMP-gated channels in the outer segment. Black, control mouse. Red, homozygous knockout mouse lacking functional RGS9-1. Inhibiting the GTPase activity of transducin greatly slows recovery but barely affects the amplitude of the response. Modified from [13].

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